1978

The effect of temperature on the life cycle of Drosophila nebulosa

Scott S. Nagatani
University of the Pacific

THE EFFECT OF TEMPERATURE ON THE LIFE CYCLE OF *DROSOPHILA NEBULOSA*

A Thesis

Presented to

The faculty of the

Department of Biological Sciences

University of the Pacific

In Partial Fulfillment

of the Requirements of the Degree

Master of Science

by

Scott S. Nagatani

May 1978

This thesis, written and submitted by

Scott S. Nagatani

is approved for recommendation to the Committee on Graduate Studies, University of the Pacific.

Department Chairman or Dean:

F. R. Hunter

Thesis Committee:

Alice S. Hunter Chairman

F. R. Hunter

Dallas McNeal

Dated May 1, 1978

ACKNOWLEDGEMENTS

I would like to gratefully acknowledge Dr. Alice S. Hunter for her assistance and guidance during the research and writing of this paper. I would like to thank Dr. and Mrs. Lee Christianson for their time and effort in helping me with the statistical analysis, and also Graduate students Stan Carson, Jim Redman, John Shaffer, and Jim Stavosky for aiding me in the accumulation of the data. Finally I would like to thank God for my wonderful, patient, and loving helpmate, Sharon; for my always encouraging parents; and for the peace He gives me through His Son, Jesus Christ.

TABLE OF CONTENTS

	Page
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION	1
MATERIALS AND METHODS	14
RESULTS	18
DISCUSSION	48
SUMMARY	60
LITERATURE CITED	61

LIST OF TABLES

		Page
Table 1.	Duration of the stages of the life cycle of *D. nebulosa* at 20 C.	21
Table 2.	Duration of the stages of the life cycle of *D. nebulosa* at 25 C.	22
Table 3.	Duration of the stages of the life cycle of *D. nebulosa* at 30 C.	23
Table 4.	Duration of the overall life and/or egg to eclosion period at different temperatures in *Drosophila* species. . .	52
Table 5.	Q_{10} of the overall life cycle and/or egg to eclosion period for different temperature ranges in *Drosophila* species	53

LIST OF FIGURES

		Page
Figure 1.	Dice-Leraas diagram (Simpson, Roe, and Lewontin, 1960) of duration of overall life cycle at different temperatures in *D. nebulosa*	24
Figure 2.	Regression line of duration of overall life cycle at different temperatures in *D. nebulosa* (Reciprocal transformation of both duration and temperatures was necessary).	26
Figure 3.	Dice-Leraas diagram of duration of "eclosion to sexual maturity" stage at different temperatures in *D. nebulosa*	28
Figure 4.	Regression line of duration of "eclosion to sexual maturity" stage at different temperatures in *D. nebulosa* (No transformation was necessary. The dashed lines indicate the 95% prediction limits about the regression line.).	30
Figure 5.	Dice-Leraas diagram of duration of "sexual maturity to egg" stage at different temperatures in *D. nebulosa*. .	32
Figure 6.	Dice-Leraas diagram of duration of "egg to larva" stage at different temperatures in *D. nebulosa*.	34
Figure 7.	Regression line of duration of "egg to larva" stage at different temperatures in *D. nebulosa* (No transformation was necessary. The dashed lines indicate the 95% prediction limits about the regression line.).	36
Figure 8.	Dice-Leraas diagram of duration of "larva to pupa" stage at different temperatures in *D. nebulosa*.	38

Page

Figure 9. Regression line of duration of "larva to pupa" stage at different temperatures in D. nebulosa (Reciprocal transformation of both duration and temperature was necessary). 40

Figure 10. Dice-Leraas diagram of duration of "pupa to eclosion" stage at different temperatures in D. nebulosa 42

Figure 11. Regression line of duration of "pupa to eclosion" stage at different temperatures in D. nebulosa (Reciprocal transformation of both duration and temperature was necessary.) 44

Figure 12. Summary of duration of the stages of the life cycle at 20 C, 25 C, and 30 C in D. nebulosa (Mean, range, 2 standard errors of the mean, and N are shown). 46

INTRODUCTION

Drosophila is the genus of pomace flies or small fruit flies, in the family Drosophilidae and in the order Diptera. The genus is worldwide in distribution with the majority of the species tropical or semitropical. In colder areas, i.e., mountain masses and the Arctic regions, only a few species are to be found. Thus, only 9 species have been found in Alaska (586,400 sq. mi.) while there are at least 120 species in El Salvador (8,260 sq. mi.), and more than 400 in Hawaii (6,435 sq. mi.) (Spieth and Heed, 1972).

The vast majority of Drosophila are inhabitants of woodlands. A relatively small number of species, the bulk of which are cactus-dependent members of the repleta group, have invaded arid and semi-arid areas. None seems to be found in grasslands and tundra. The adults are diurnal and display relatively short periods of activity during each morning and evening in their visits to feeding and courtship sites. There are species-specific differences, but in cooler climates and arid areas the activity periods are usually shorter and more precisely delineated than in tropical rain forests. During the remainder of the day and night the individuals are scattered and hidden in secluded sites where they appear to remain relatively inactive. A few species, mostly the cosmopolitan ones, tend to persist for prolonged periods on

or near the food sites, especially in situations such as tomato fields, orchards, large semicovered garbage cans, and waste fruit dumps (Spieth and Heed, 1972).

The order Diptera is characterized by holometabolous development (i.e., complete metamorphosis). In this type of development the immature and adult stages are usually quite different in form, often live in different habitats, and may have very different habits. From the egg, hatches a wormlike larva which, in Drosophila, molts twice by shedding the cuticle, mouth hooks, and spiracles. The period of growth before each molt is called an instar, and there are 3 of these. The cuticle of the third instar hardens and darkens to become the puparium in which metamorphosis occurs. The pupa begins to darken just prior to the emergence of an adult fly. Shortly before emergence, the folded wings appear as two elliptical bodies, and the pigment in the eyes is visible through the puparium. When metamorphosis is complete, the adult emerges (ecloses) by forcing its way through the anterior end (operculum) of the puparium. After eclosion, the fly becomes sexually mature and mates. The female then lays her eggs, and the cycle begins again (Flagg, 1973).

An important environmental factor which affects the length of this cycle is temperature. According to Suzuki (1970), the life cycle of D. melanogaster from egg to fertile adult required 22-28 days at 17 C, 11-12 days at 22 C, and 7-8 days at 29 C. At 25 C, Powsner (1935) found that D.

melanogaster required 8 days plus 18 hours from egg-laying to emergence of adults. Budnik, Koref-Santibanez, and Brncic (1971) found that the developmental rate from egg to adult in D. pavani was 40 days at 16 C and 18 days at 25 C. Ray (1960) found that the egg to adult period for D. pseudo-obscura, D. persimilis, D. equinoxalis, and D. willistoni was 31-34 days at 16 C and 16-22 days at 19 C. Also, at 24 C, the first 2 species required 18 days while the last 2 species required 10-11 days. From these results it is obvious that the length of the life cycle decreases with increasing temperature.

Since the length of the life cycle depends on many different metabolic functions, it is not surprising that an effect of temperature on other rate functions in Drosophila has been demonstrated. Insects, in general, are considered poikilotherms as opposed to homeotherms which include birds and mammals. Poikilothermic animals, which include the overwhelming majority of species, have a relatively variable body temperature and are ectothermic in that the heat that determines the body temperature is largely obtained from the environment rather than from their own oxidative metabolism. On the other hand, homeotherms have a relatively constant body temperature and are endothermic. Endothermic animals produce sufficient heat by their own oxidative metabolism and have a sufficiently low thermal conductance so that the heat which contributes to their body temperature is largely

derived from their own oxidative activity (Gordon, et. al., 1972).

A wide variety of animals are active in air temperatures between 0 and 35 C. It is, therefore, profitable to examine the response of oxygen consumption and body temperature of a hypothetical homeotherm and a hypothetical poikilotherm to this range of temperatures. Over the restricted temperature range being considered, the hypothetical poikilotherm has a body temperature that is indistinguishable from that of the environment, so it expends virtually no energy on thermoregulation. On the other hand, the hypothetical homeotherm holds its body temperature at some closely regulated level which is independent of environmental temperature. To maintain this independence, it must continuously expend energy. Consquently, if oxygen consumption is plotted against environmental temperature, the responses of the poikilotherm and the homeotherm follow different patterns. The poikilotherm's rate of oxygen consumption behaves like that of an *in vitro* biochemical system and doubles for each 10 C increase in temperature which is in general conformance with van't Hoff's rule. In contrast, the oxygen consumption of the homeotherm decreases linearly with increasing environmental temperature to some critical point and then is independent of environmental temperature over a considerable range (Gordon, et. al., 1972).

Two ecological rules that state a relationship between environmental factors, such as temperature, and the morpho-

logical characteristics of a species are Bergmann's Rule and Allen's Rule. Bergmann's Rule states that the body size of geographical races of a homeothermic species is smaller in the warmer parts of the species' range than in the colder parts of the range. Allen's Rule says that protruding body parts are relatively shorter in the cooler parts of the range of a species. The validity of these rules was well established for birds and mammals, but Ray (1960) wanted to see to what extent they operate in poikilotherms. Of the 17 poikilothermic species studied in his experiment, 4 were Drosophila species. These were D. willistoni, D. equinoxialis, D. pseudoobscura, and D. persimilis. For each species, 3 measurements were made: wing length from the base of the alula to the tip, the length of the second tibia, and the total wet weight. These were measured at 2 temperatures with an approximate difference of 10 C. It was found that in all 4 species, at the lower temperature the wing and tibia lengths were both relatively less while the total wet weight was significantly greater. Thus, Bergmann's and Allen's Rules apply to the 4 species of Drosophila studied.

Others have also found Bergmann's and Allen's Rules to apply to Drosophila. Alpatov and Pearl (1929) noted long ago that D. melanogaster reared at 18 C is distinctly larger in a series of bodily dimensions than when reared at 28 C. Pantelouris (1957) confirmed the existence of a difference in thorax size between the male offspring of reciprocal crosses of large and small selected strains of D. melanogaster.

A similar and larger difference was observed in wing length. Pantelouris found that these differences could be enlarged to a varying extent by lowering the temperature during the larval or pupal or both stages. The percentage of length increase was approximately the same for the thorax and the wing when the temperature was kept low during the larval stage only, but the wing increase became 6 times larger than the thorax increase if the temperature was lowered during the pupal stage only. Pantelouris also reported that the wing-length to thorax-length index increased with the lowering of temperature. Tantawy and Mallah (1961) also noted that the wing and thorax lengths of D. melanogaster and D. simulans were greater at lower temperatures, decreasing gradually with increasing temperature. Also, the wing-thorax ratio was about 2 under optimum conditions, increasing at lower temperatures and decreasing at higher ones. Working with 4 species, D. melanogaster, D. viracochi, D. pseudoobscura, and D. willistoni, Burr and Hunter (1969) concluded that in general, the dry weight was higher at the lower temperature, but the percentage water did not differ with the temperature or species.

In addition to body size, the results of Tantawy and Mallah (1961) show clearly that the percentage of emergence as well as sex-ratio can be altered by changing temperature conditions. The offspring of natural populations of D. melanogaster and D. simulans captured from different

geographical regions in Lebanon, Egypt, and Uganda, were raised under a variety of temperatures ranging from 10 C to 31.5 C in D. melanogaster and from 10 C to 30.5 C in D. simulans. It was found that at the 2 extremes of temperature the percentage of emergence was lower, with the females significantly more numerous than males. In contrast, at milder and optimum conditions the percentage of emergence was significantly higher with equal numbers of males and females.

It is also known that high temperatures cause sterility in Drosophila. This was first noted by Northrop (1920) when he demonstrated in D. melanogaster that at a point 5 C to 9 C above the normal optimum of 24 C, no offspring were produced after one generation. Young and Plough (1926) later found that high temperature had a differential effect on the germ cells of Drosophila such that males were rendered completely sterile at a point where females were still completely fertile. This effect on the males was permanent as long as this temperature was maintained, but most males recovered normal fertility after being returned to the optimum temperature. Young and Plough also found that exposures to 31 C of over 10 days greatly increased the number of flies which were permanently sterilized. Examination of the testes of sterile males showed that high temperature caused loss of motility of the sperm with progressive aggregation and degeneration. Few or no spermatozoa were found

in the lower end of the testis and the vas deferens. Although such males continued to copulate with females at high temperature, no sperm passed into the ventral receptacles of the females. David, Arens, and Cohet (1971) found that a thermosensitive period corresponding to the beginning of spermiogenesis appears to exist in male gametogenesis and to last 1 or 2 days. If this sensitive period occurs at 30 C, irreversible lesions in the geminal cells take place. Tantawy and Mallah (1961) found that complete sterility occurred at 31.5 C in populations of D. melanogaster and at 30.5 C in D. simulans.

Lauge (1972) studied the effect of elevated temperatures on the life span and reproduction rate of D. melanogaster. D. melanogaster, strain Oregon RC flies, reared for several years in the laboratory at 25 C, were subjected to a temperature of 30 C which resulted in no progeny after the second generation. Both generations raised at 30 C had a shortened imaginal (adult) life compared to flies raised at 25 C. While the life span of males was equal in both generations, that of the females was shorter in the second, i.e., 4.9 days, compared to 8.3 days for the first generation. The normal life span at 25 C was 28.4 days for females and 40.3 days for males. Also, females raised at 30 C had a reproduction rate 92 times lower than females raised at 25 C.

Smith (1958) found correlations between temperature and egglaying and their effects on the longevity of D.

subobscura. "Ovariless" females and virgin females live significantly longer than normal mated females. The life span of "ovariless" females at 20 C was not altered by exposure to 30.5 C. Smith concluded that egg-laying accelerates the aging of females at 20 C, and that the prolongation of life of females exposed to 30.5 C is due to the reduction in the rate at which such females subsequently lay eggs. Alpatov (1932) found that the temperature during development of D. melanogaster greatly influenced the time when the reproductive period began.

Resistance and acclimation to dry heat was studied in several Drosophila species collected in Puerto Rico (Levins, 1969). Adaptation was allowed to take place at temperatures within the normal range for the region from which the flies were taken, and the results were measured in duration of survival at 37 C - 38 C, a stress condition which nevertheless occurs in nature. By choosing these conditions, the effects of heat were confounded with dessication. Levins found that physiological acclimation did not occur in narrow-niched species such as D. prosaltans but did occur in broad-niched species such as D. melanogaster which depends more on individual flexibility and less on genetic differences among populations for adaptation to different climates. Levins feels that this work may be extrapolated to the wild, and such acclimation may permit feeding in dry places without immediate dessication. Also, D. willistoni, a moderately broad-niched species, did not acclimate nor show much genetic

variation. It was suggested by Levins that this species avoided dessication stress behaviorally.

Druger (1962) studied the effect of low temperature on development in D. pseudoobscura. Eggs were placed at 5 C and after 178 days at this low temperature, the surviving larvae were transferred to 19 C. Although mortality was high, pupae formed and, within 10 days, adults emerged. These were mated, and fertile offspring resulted. Druger concludes that since development could be extended for 6 months (and perhaps longer) by low temperature, this suggests that this phenomenon may be possible in natural populations of D. pseudoobscura during winter months. Crumpacker and Marinkovic (1967) studied the resistance of D. pseudoobscura to cold temperature. Adults, pupae, larvae, and eggs were exposed to temperatures of 16 C, 5 C, -3 C, and -10 C for periods of 1, 7, and 21 days. Resistance to cold temperature was measured in terms of survival of adults or survival and subsequent development of nonadult stages. It was found that adults were more resistant to cold stress than were larvae or pupae, which in turn were more tolerant than eggs.

Tantawy and Mallah (1961) studied natural populations of D. melanogaster and D. simulans in the Middle East and surrounding areas to test a hypothesis concerning the nature of developmental homeostasis. The hypothesis was that progeny of euryokous populations (i.e., populations that have a wide tolerance for various environmental factors) were better

able to withstand the upper and lower limits of experimental laboratory conditions than progeny of stenokous populations. It was found that the euryokous population was more viable over a wide range of temperature while the stenokous population was superior only at one temperature. Hunter (1964) hypothesized that species which live in a wide range of climates (eurytherms) may have a superior capacity for adaptation to different temperatures than do species which live in a narrow range of climates (stenotherms). D. melanogaster, D. hydei and D. immigrans were chosen as examples of eurythermal species while D. pseudoobscura, D. viracochi, and D. willistoni were chosen as examples of stenothermal species (Hunter, 1964, 1965, 1966, and 1968). The oxygen consumption, as an indication of the rate of respiration, was measured for adults grown at different temperatures. It was found that D. melanogaster females, and both sexes of D. hydei and D. immigrans when acclimated at 15 C, had a higher rate of respiration than that of flies acclimated at 25 C when the rate of oxygen consumption was measured at the intermediate temperature of 20 C. According to Prosser and Brown (1961), the normal pattern of temperature adaptation is such that cold-acclimated organisms have a higher rate of respiration (rate function) at an intermediate temperature than do the warm-acclimated ones. Thus, the 3 eurythermal species exhibited temperature adaptation. It was also found that the 3 stenothermal species when acclimated

at 15 C, either had an equal or lower rate of respiration than that of flies acclimated at 25 C when the rate was measured at 20 C. These results for the stenothermal species suggest lack of temperature adaptation according to Prosser and Brown (1961). Thus, the results for all 6 species studied are in conformity with the proposed hypothesis (Hunter, 1964).

In addition to oxygen consumption, other rate functions have been studied to learn more about the mechanisms of temperature adaptation in Drosophila. The enzyme activity of glutamate-aspartate transaminase was studied by Burr and Hunter (1970) to attain information on temperature adaptation at the enzyme level in 2 eurythermal species, D. melanogaster and D. immigrans, and 2 stenothermal species, D. pseudoobscura and D. willistoni. Each of the 4 species was acclimated at 15 C and 25 C, and their enzyme activities were measured at 20 C. D. melanogaster females and D. immigrans males and females displayed temperature adaptation in that flies grown at 25 C had lower transaminase activity than did those grown at 15 C when measured at 20 C. However, glutamate-apartate transaminase activity did not vary with acclimation temperature in D. pseudoobscura and D. willistoni. These results support the hypothesis that eurythermal species of Drosophila have greater capacity for physiological adaptation than do stenothermal species.

It is the purpose of this research to study the effect of temperature on the life cycle of D. nebulosa. D. nebulosa

is considered a stenothermal species from a warm environment and has been reported in Texas and Florida, the West Indies, Mexico, Central America, and as far south as Brazil (Patterson, 1943, Heed, 1956, Garcia and Suarez, 1962, and Hunter and Navarro, 1969). According to the hypothesis of Hunter (1964), the capacity of this species for adaptation to different temperatures would not be expected to be as great as that of a eurythermal species. Stenothermal species are relatively limited by the environmental temperature, and therefore, one would expect a marked decrease in the length of the life cycle with increasing temperatures. On the other hand, eurythermal species are relatively independent of the environmental temperature, so a relatively less decrease in the length of the life cycle with increasing temperature would be expected.

MATERIALS AND METHODS

D. nebulosa were collected in Barquisimeto, Venezuela over various types of fruit. They have been maintained in the laboratory at 25 C previous to these studies.

Stocks were allowed to develop in incubators at temperatures of 20±1 C, 25±1 C, and 30±1 C in several 240 ml glass stock jars with sterile cotton stoppers. Each stock jar contained 12 g of Instant Drosophila Medium (#67-5002, Formula 4-24, Carolina Biological Supply Company) mixed with 34 ml of water. The flies were exposed to light daily from 8:00 A.M. to 8:00 P.M.

Eighty ml plastic vials with sterile cotton stoppers were utilized in these experiments. The contents of each experimental vial included 6 g of Instant Drosophila Medium, 17 ml of water, .2 g of Fleischmann's Active Dry Yeast as additional food, and a few drops of Schilling's Green Food Coloring to provide a contrasting background for the light-coloured Drosophila eggs.

Virgin flies of both sexes were collected from the stock jars of one incubator, e.g., 20 C, approximately every 12 hours around 8:00 A.M. and 8:00 P.M. These flies were then etherized and sexed. Six females and six males were put in each experimental vial which was then placed into the same incubator from which the stock jars came. The

light cycle mentioned above was maintained throughout the experiment. Occasionally, due to a small supply of available virgins, less than 6 females and 6 males were placed in an experimental vial, e.g., 6 females and 5 males or 5 females and 4 males, etc. This alteration was assumed to have no effect on the results. Each experimental vial was examined daily between 8:00 A.M. and 10:00 A.M. to note the different stages of the life cycle of the offspring with the aid of a dissecting microscope. The day and the approximate time of the first appearance of eggs, larvae, pupae, and finally, emerging adults were recorded. This procedure was continued until complete data for at least 20 experimental vials were collected at each of the 3 temperatures studied.

After eclosion, the flies reach sexual maturity and mate. In the results, this is designated as the first stage of the life cycle and called the "eclosion to sexual maturity" stage. The length of this stage was measured by an entirely separate procedure from that described above and with a different group of flies. Stocks of D. nebulosa were maintained as previously described except that sterile foam rubber stoppers instead of sterile cotton stoppers were used at 30 C.

Virgin flies of both sexes were collected in the same manner stated earlier, and for each of the 3 temperatures 6 groups of 4 vials were studied. In groups 1-6, the males were removed from the vials after 12, 24, 36, 48, 60, and

72 hours of incubation, respectively. In the first group, for example, the flies in each vial were removed, etherized, and sexed after 12 hours of incubation. Only the females were then returned to their respective vials. If copulation had occurred within the initial 12 hour period when the 2 sexes were together, then the subsequent presence of larvae and/or pupae would be observed. The presence of eggs do not necessarily indicate that copulation had occurred since female fruit flies also lay unfertilized eggs. This procedure was conducted for all 6 groups at the 3 temperatures studied.

The second stage is labelled "sexual maturity to egg" and is calculated by subtracting the mean of the "eclosion to sexual maturity" stage from each individual value of the "eclosion to egg" stage which was derived in the initial procedure where the first appearance of the different life cycle stages was noted. Thus, the "sexual maturity to egg" stage is the result of combining the data from the 2 methods described.

A second part of this experiment was to study the resistance of D. nebulosa to a cold temperature of 15 C. Stocks of flies were maintained in the 25±1 C incubator with a daily light exposure from 8:00 A.M. to 8:00 P.M. Virgin males and females were again collected in the same manner described previously, placed in experimental vials at 15±1 C in an incubator which was also on a daily light cycle. These

were then observed to see if mating, egg-laying, and development of progeny occurred. The resistance of the various stages of the life cycle to cold stress was also studied. Eggs, larvae, and pupae were transferred from 25 C to 15 C to learn whether or not development to adult would continue. In both of these cold temperature experiments, only qualitative observations were made.

RESULTS

As shown in Tables 1-3 and Figure 1, the mean duration of the overall life cycle of *D. nebulosa* is 21.1±0.3 days at 20 C, 13.6±0.2 days at 25 C, and 11.9±0.2 days at 30 C. The "2-sided" Student's t-test with P=0.05 showed that all 3 means are significantly different. A reciprocal transformation of both duration and temperature was necessary to calculate the regression line shown in Figure 2. The result of the regression line is the formula $1/D = 0.16 - 2.17\ (1/T)$ which allows one to predict the duration, D, of the overall life cycle at any temperature, T. Because of the data, the regression line and prediction limits coincide.

The mean duration of the "eclosion to sexual maturity" stage as shown in Tables 1-3 and Figure 3, is 2.8±0.2 days at 20 C, 2.4±0.1 days at 25 C, and 2.0 days at 30 C. There is no estimate of variability at 30 C since the sample size was only 1. Because of this, the t-test was not calculated for 30 C, but it was found that the means at 20 C and 25 C were not significantly different. No transformation was necessary to determine the regression line which is shown in Figure 4. The formula which allows the prediction of the duration of this stage at a temperature is $D = 4.48 - 0.08\ T$, with P = 0.05.

The mean duration of the "sexual maturity to egg" stage is 0.8±0.2 days at 20 C, 0.3±0.1 days at 25 C, and 0.4±0.1 days at 30 C, and this is shown in Tables 1-3 and Figure 5. Negative values appear in the figure because this value is the result of combining the data from the 2 methods described in the Materials and Methods Section. Using the Student's t-test, it was found that the means at 20 C and 25 C differ significantly, whereas each of these means do not differ significantly from the mean at 30 C. Since the values at 30 C were derived from the single value of the "eclosion to sexual maturity" stage, and since a sample size of 1 gives no estimate of the variability, no line of regression for the "sexual maturity to egg" stage was calculated. Consequently, a prediction formula was not determined.

As shown in Tables 1-3 and Figure 6, the mean duration of the "egg to larva" stage is 4.0±0.4 days at 20 C, 4.0±0.3 days at 25 C, and 2.7±0.2 days at 30 C. The means at 20 C and 25 C are not significantly different, but both of these means are significantly different from the mean at 30 C. No transformation of the data was required in calculating the regression line which is shown in Figure 7. The prediction formula is $D = 6.98 - 0.14\ T$ with $P = 0.05$.

The mean duration of the "larva to pupa" stage is 5.4±0.5 days at 20 C, 3.1±0.4 days at 25 C, and 2.8±0.2 days at 30 C as shown in Tables 1-3 and Figure 8. The mean at 20 C differs significantly from the means at 25 C and 30 C,

while the 2 latter means are not significantly different. A reciprocal transformation of both duration and temperature was necessary to calculate the line of regression shown in Figure 9. The prediction formula is D = 0.95 - 13.31 (1/T), and again due to the data, the regression line and prediction limits coincide.

As shown in Tables 1-3 and Figure 10, the mean duration of the "pupa to eclosion" stage is 8.4±0.2 days at 20 C, 4.6±0.1 days at 25 C, and 4.2±0.1 days at 30 C. All 3 means are significantly different. A reciprocal transformation of both duration and temperature was again necessary in the calculation of the regression line shown in Figure 11. The prediction formula is 1/D = 0.48 - 6.99 (1/T) with the regression line and prediction limits coinciding.

Figure 12 is a summary of the durations of each stage at 20 C, 25 C, and 30 C. It is clear that the general trend for the length of each stage of the life cycle is to decrease with an increase in temperature.

TABLE 1

Duration of the Stages of the Life Cycle of *D. nebulosa* at 20 C

Stage	N	Mean (days)	Standard Error of mean (days)	Standard Deviation (days)	% of overall life cycle
Eclosion to sexual maturity	3	2.8	0.2	0.3	13.3
Sexual maturity to egg	21	0.8	0.2	1.1	3.8
Egg to larva	21	4.0	0.4	2.1	19.0
Larva to pupa	22	5.4	0.5	2.4	25.6
Pupa to eclosion	22	8.4	0.2	0.9	39.8
Overall life cycle	22	21.1	0.3	1.4	---

TABLE 2

Duration of the Stages of the Life Cycle of *D. nebulosa* at 25 C

Stage	N	Mean (days)	Standard Error of mean (days)	Standard Deviation (days)	% of overall life cycle
Eclosion to sexual maturity	8	2.4	0.1	0.4	17.6
Sexual maturity to egg	27	0.3	0.1	0.5	2.2
Egg to larva	21	4.0	0.3	1.5	29.4
Larva to Pupa	27	3.1	0.4	2.1	22.8
Pupa to eclosion	27	4.6	0.1	0.7	33.8
Overall life cycle	27	13.6	0.2	0.8	---

TABLE 3

Duration of the Stages of the Life Cycle of *D. nebulosa* at 30 C

Stage	N	Mean (days)	Standard Error of mean (days)	Standard Deviation (days)	% of overall life cycle
Eclosion to sexual maturity	1	2.0	---	---	16.8
Sexual maturity to egg	30	0.4	0.1	0.6	3.4
Egg to larva	29	2.7	0.2	1.3	22.7
Larva to pupa	31	2.8	0.2	1.4	23.5
Pupa to eclosion	31	4.2	0.1	0.6	35.3
Overall life cycle	31	11.9	0.2	0.9	---

Figure 1. Dice-Leraas diagram (Simpson, Roe, and Lewontin, 1960) of duration of overall life cycle at different temperatures in *D. nebulosa*

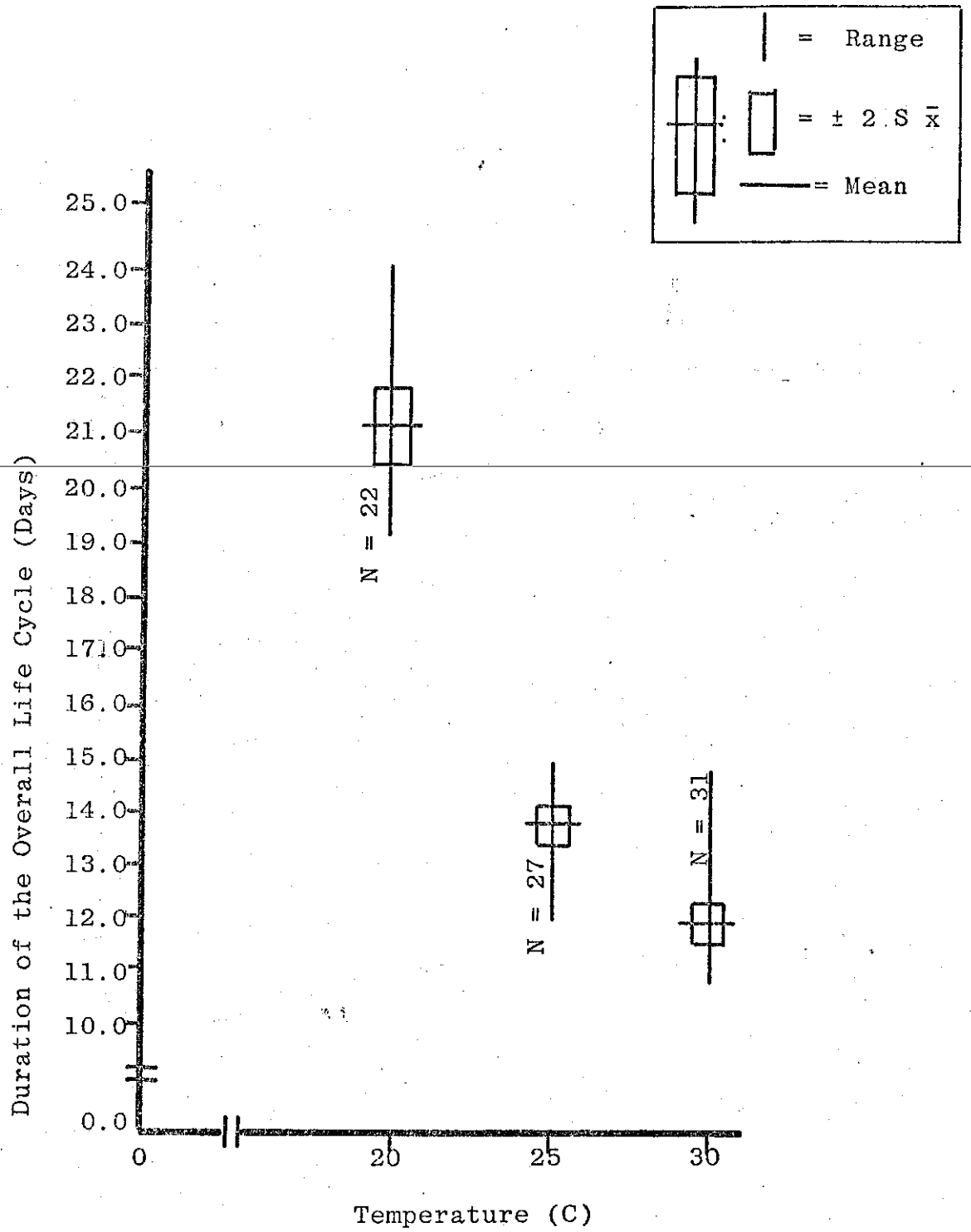

= Range
= ± 2 S x̄
= Mean
25.0
24.0
23.0
22.0
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
0.0
Duration of the Overall Life Cycle (Days)
N = 22
N = 27
N = 31
0
20
25
30
Temperature (C)

Figure 2. Regression line of duration of overall life cycle at different temperatures in D. nebulosa (Reciprocal transformation of both duration and temperature was necessary.)

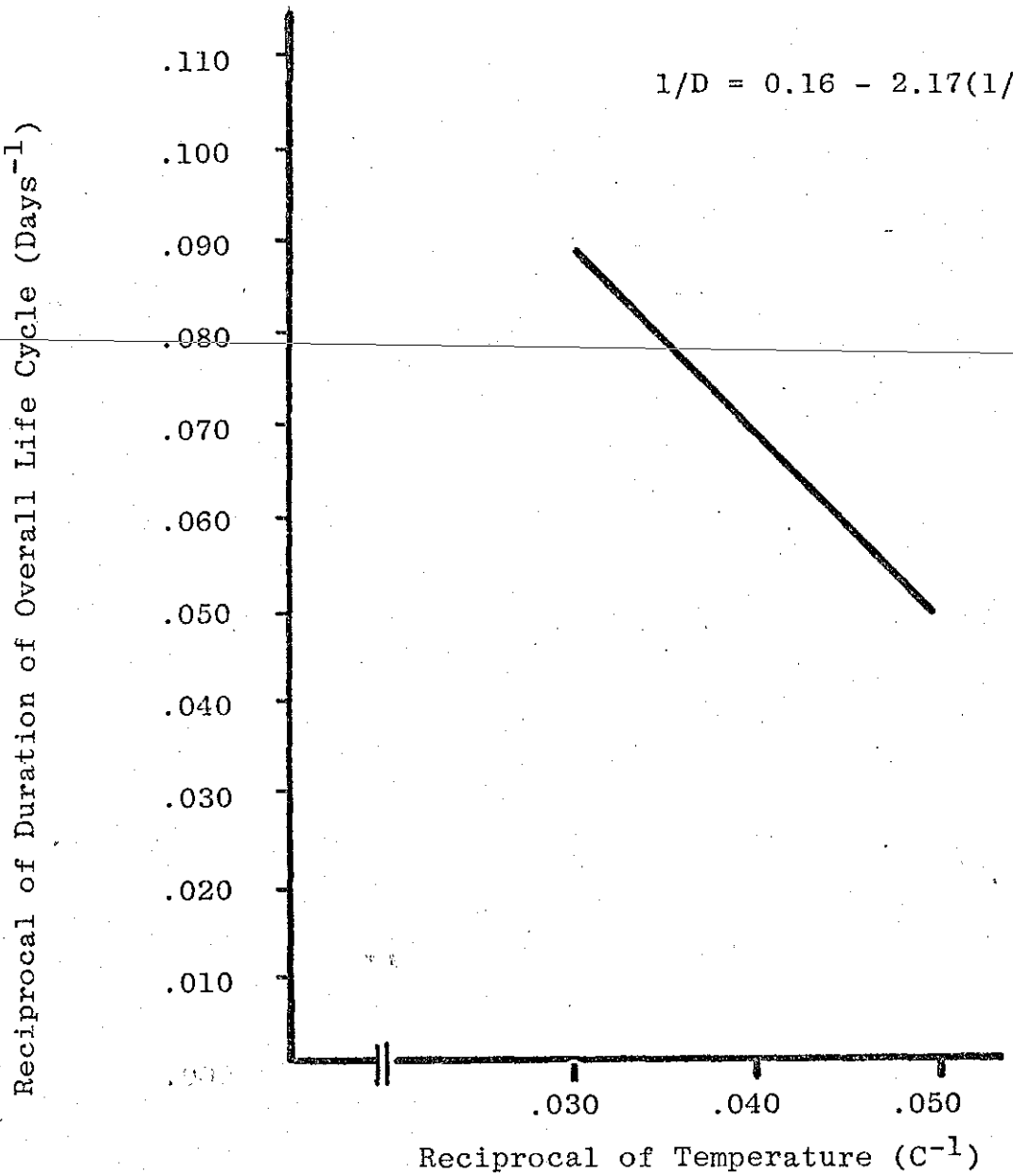

1/D = 0.16 - 2.17(1/T)
Reciprocal of Duration of Overall Life Cycle (Days^{-1})
.110
.100
.090
.080
.070
.060
.050
.040
.030
.020
.010
.030
.040
.050
Reciprocal of Temperature (C^{-1})

Figure 3. Dice-Leraas diagram of duration of "eclosion to sexual maturity" stage at different temperatures in *D. nebulosa*.

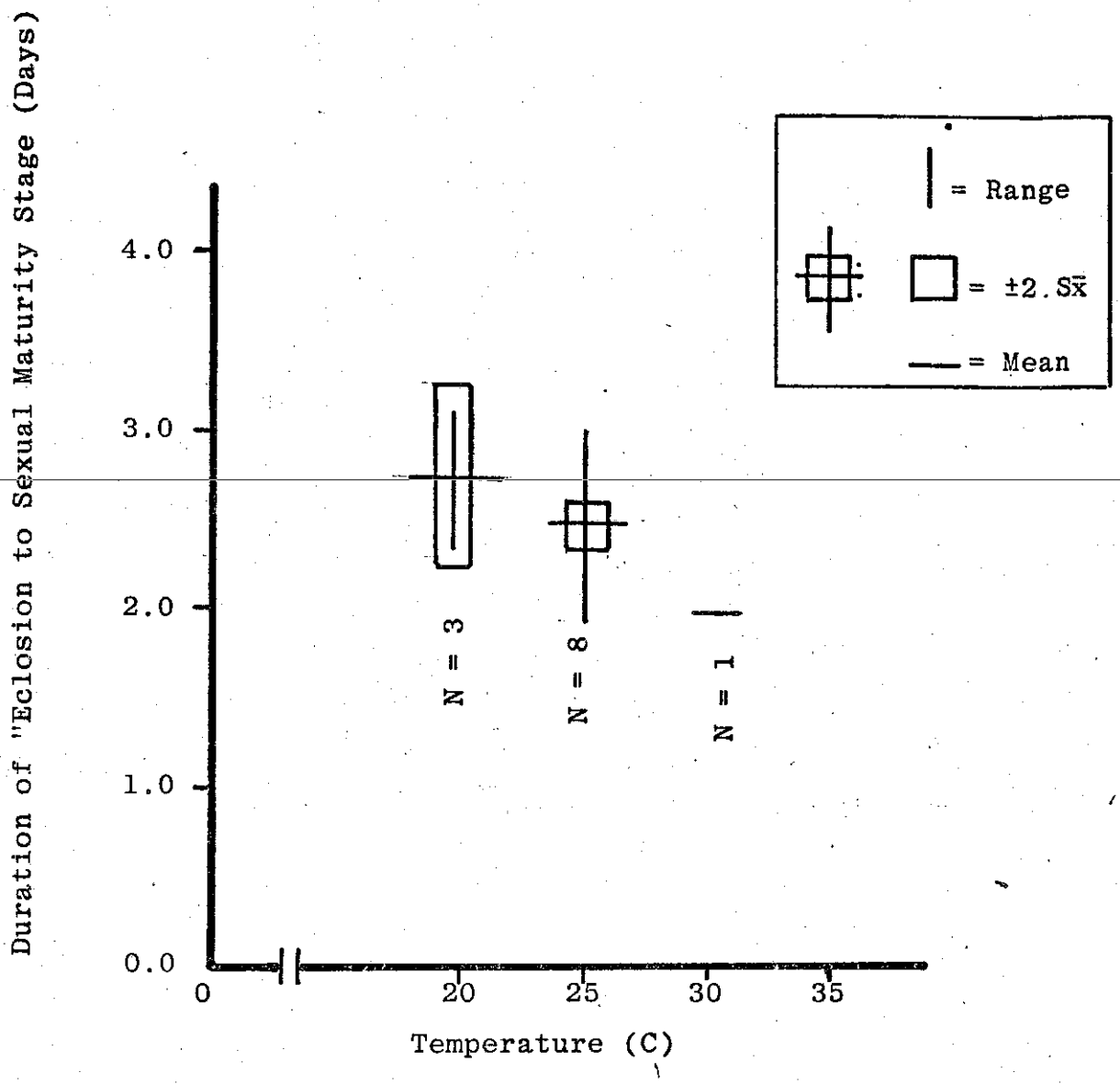

Duration of "Eclosion to Sexual Maturity Stage (Days)
4.0
3.0
2.0
1.0
0.0
0
20
25
30
35
Temperature (C)
N = 3
N = 8
N = 1
= Range
= ±2.SX̄
= Mean

Figure 4. Regression line of duration of "eclosion to sexual maturity" stage at different temperatures in D. nebulosa (No transformation was necessary. The dashed lines indicate the 95% prediction limits about the regression line.)

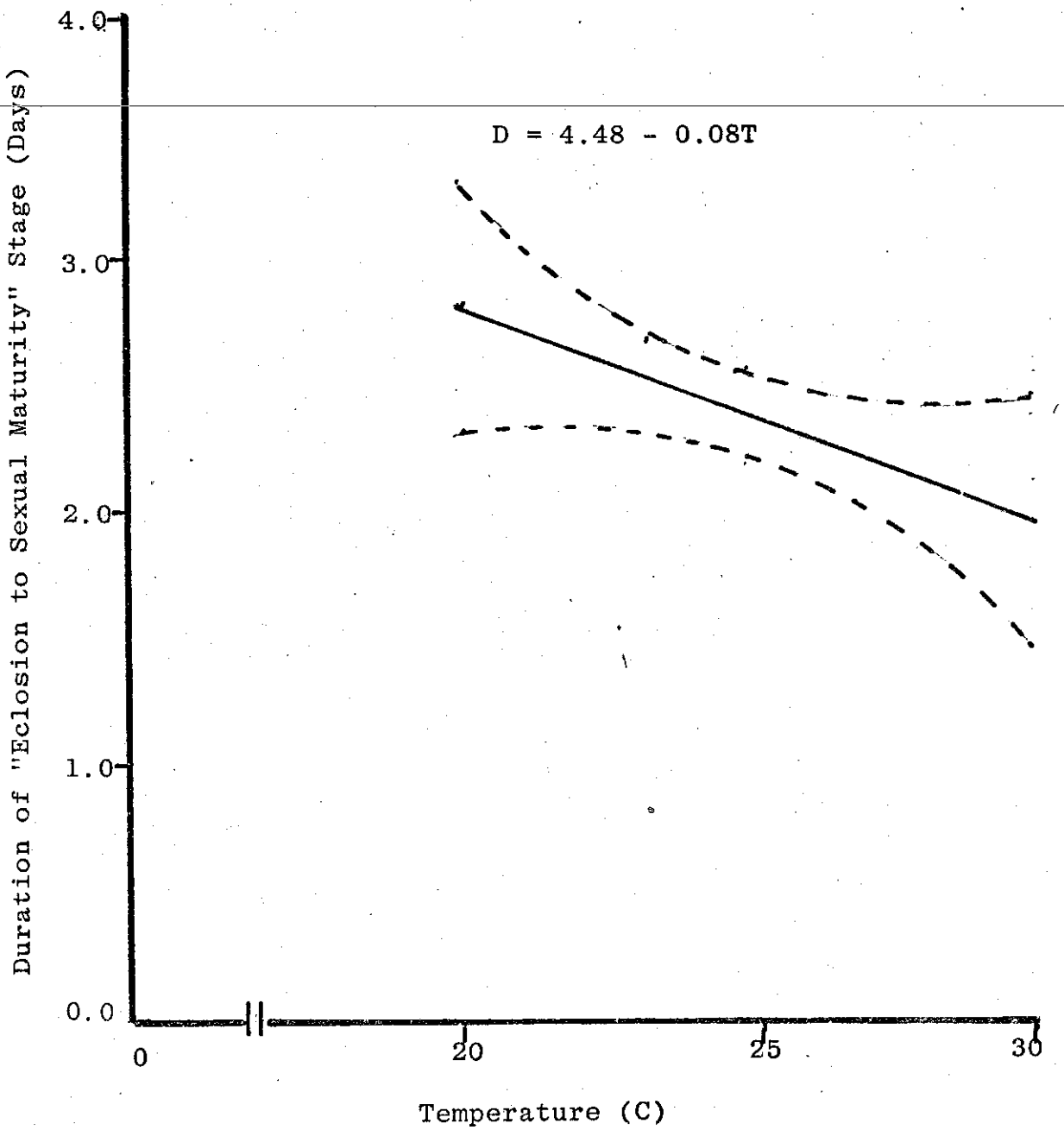

D = 4.48 - 0.08T
Duration of "Eclosion to Sexual Maturity" Stage (Days)
4.0
3.0
2.0
1.0
0.0
0
20
25
30
Temperature (C)

Figure 5. Dice-Leraas diagram of duration of "sexual maturity to egg" stage at different temperatures in D. nebulosa.

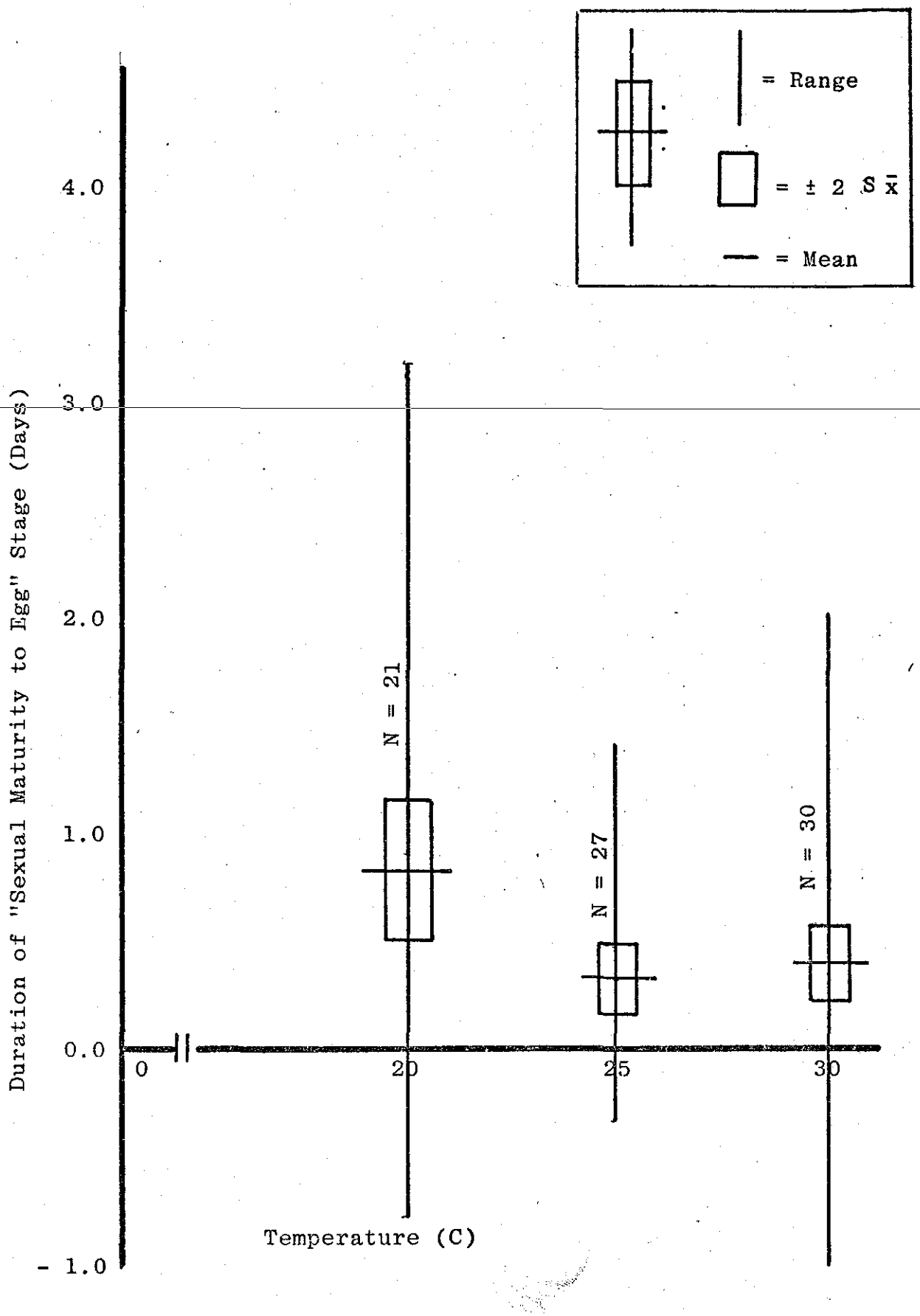

= Range
= ± 2 S x̄
= Mean
4.0
3.0
2.0
1.0
0.0
- 1.0
Duration of "Sexual Maturity to Egg" Stage (Days)
N = 21
N = 27
N = 30
0
20
25
30
Temperature (C)

Figure 6. Dice-Leraas diagram of duration of "egg to larva" stage at different temperatures in *D. nebulosa*

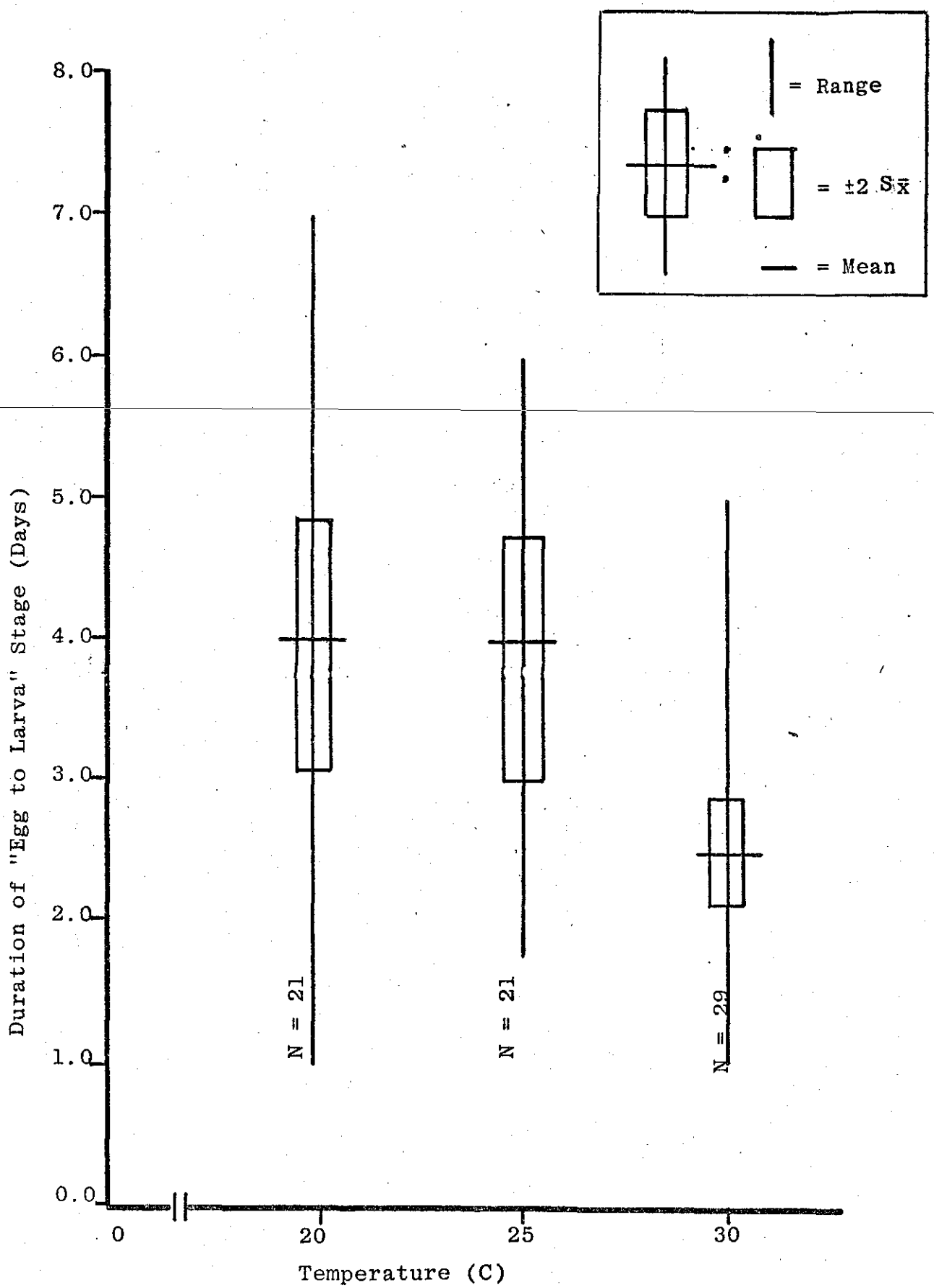

= Range
= ±2 $S_{\bar{x}}$
= Mean
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Duration of "Egg to Larva" Stage (Days)
N = 21
N = 21
N = 29
0
20
25
30
Temperature (C)

Figure 7. Regression line of duration of "egg to larva" stage at different temperatures in D. nebulosa (No transformation was necessary. The dashed lines indicate the 95% prediction limits about the regression line.)

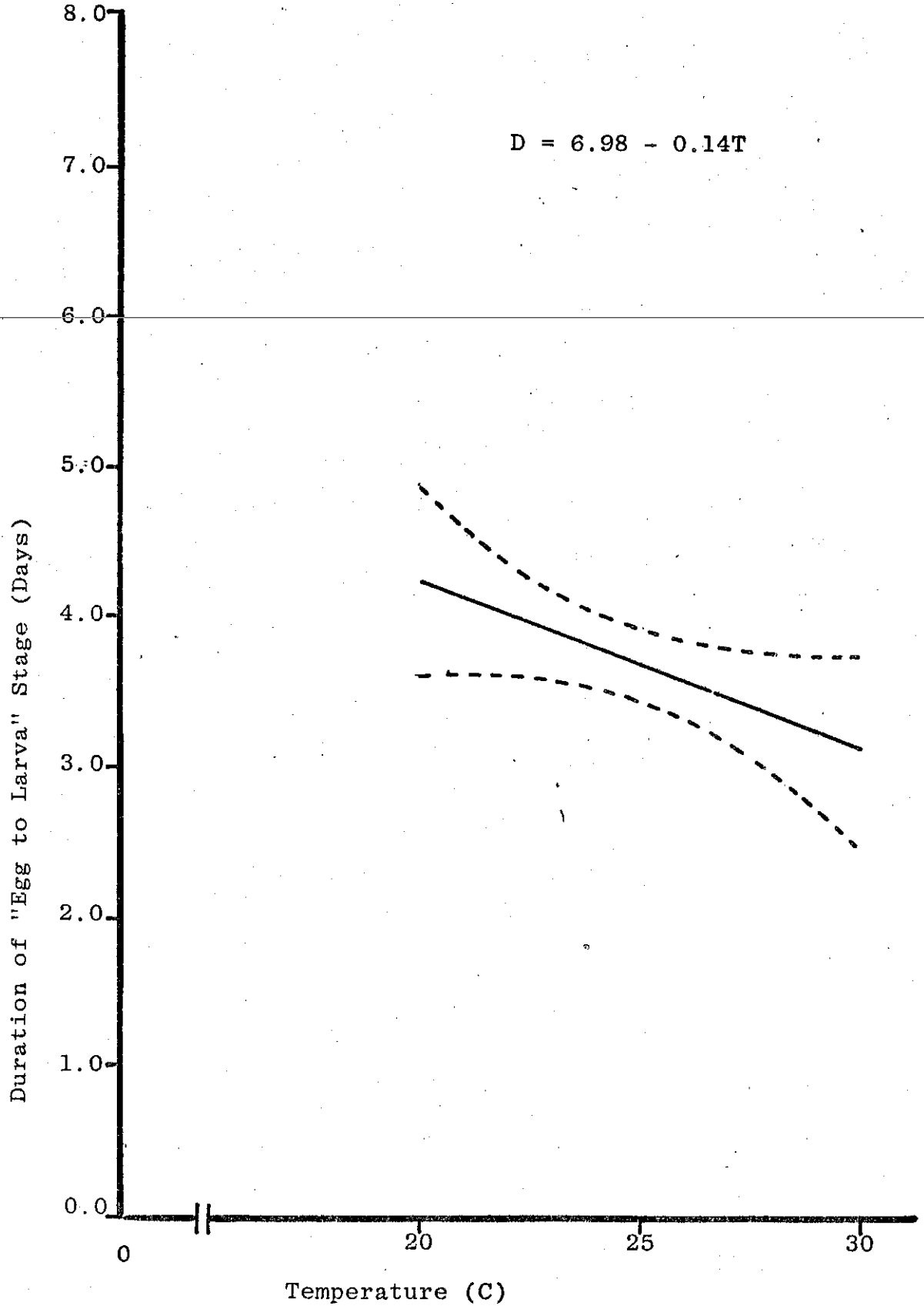

D = 6.98 - 0.14T
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Duration of "Egg to Larva" Stage (Days)
0
20
25
30
Temperature (C)

Figure 8. Dice-Leraas diagram of duration of "larva to pupa" stage at different temperatures in *D. nebulosa*.

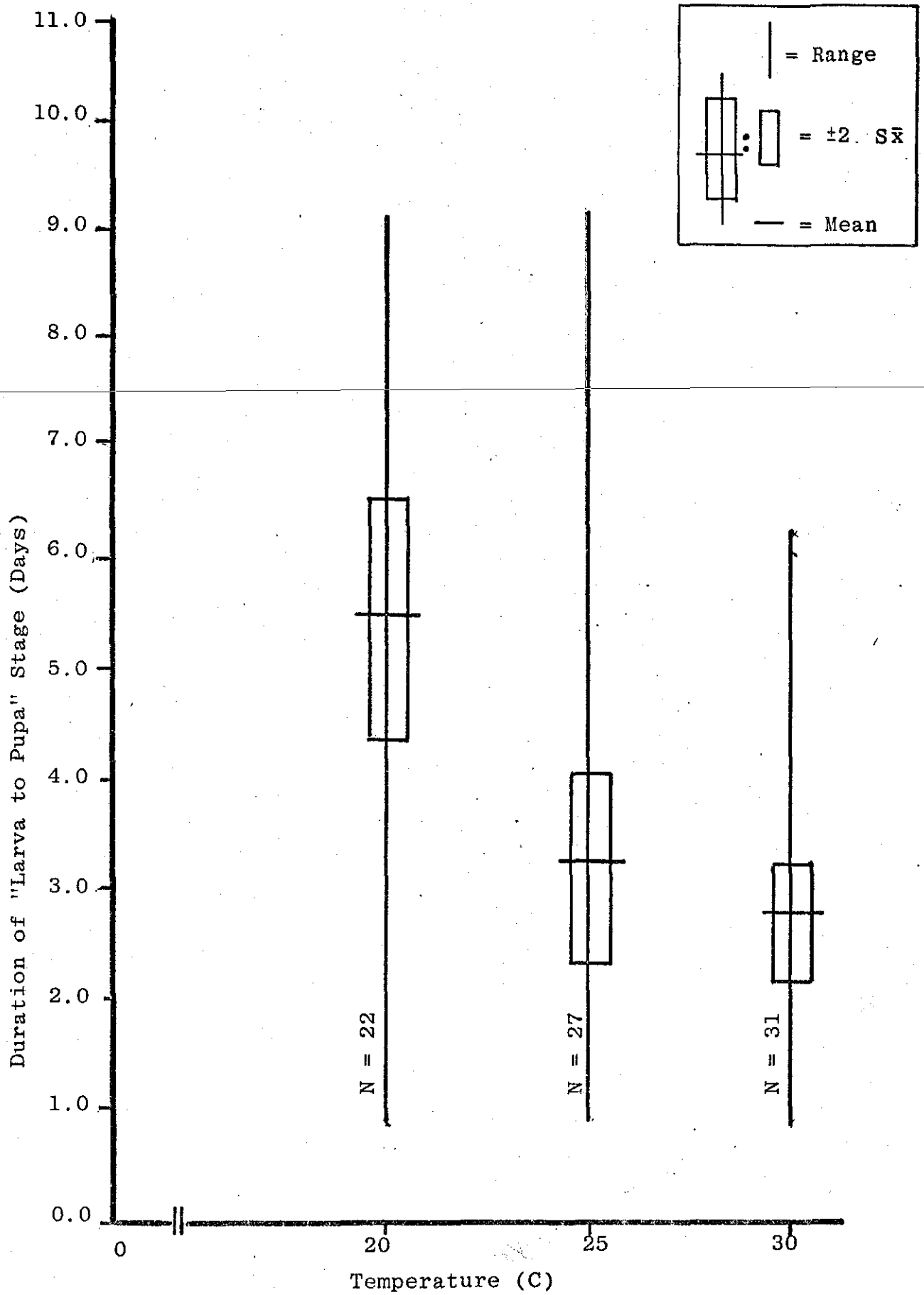

= Range
= ±2. S$\bar{x}$
= Mean
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Duration of "Larva to Pupa" Stage (Days)
N = 22
N = 27
N = 31
0
20
25
30
Temperature (C)

Figure 9. Regression line of duration of "larva to pupa" stage at different temperatures in D. nebulosa (Reciprocal transformation of both duration and temperature was necessary.)

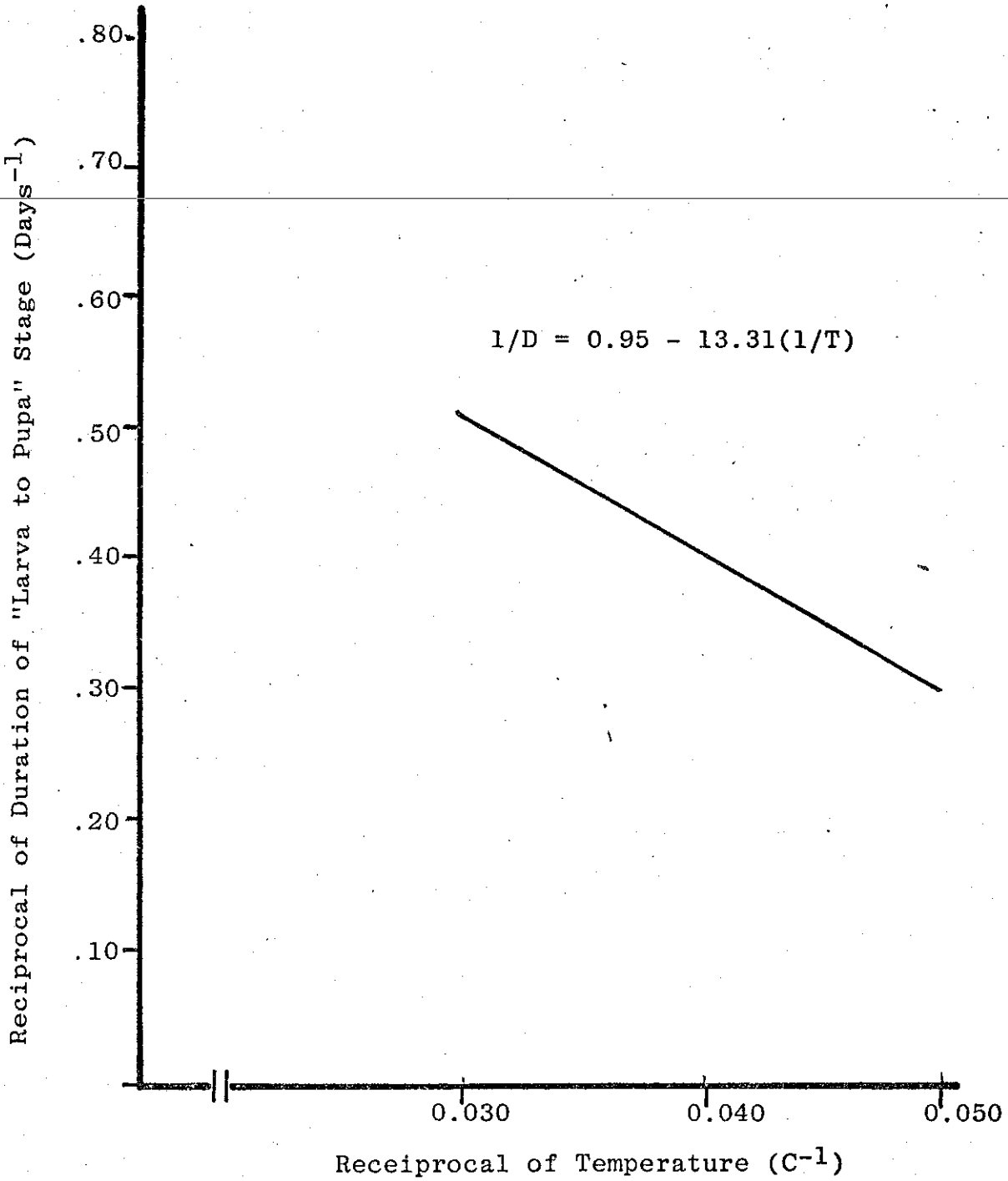

.80
.70
.60
.50
.40
.30
.20
.10
Reciprocal of Duration of "Larva to Pupa" Stage (Days^{-1})
1/D = 0.95 - 13.31(1/T)
0.030
0.040
0.050
Receiprocal of Temperature (C^{-1})

Figure 10. Dice-Leraas diagram of duration of "pupa to eclosion" stage at different temperatures in D. nebulosa

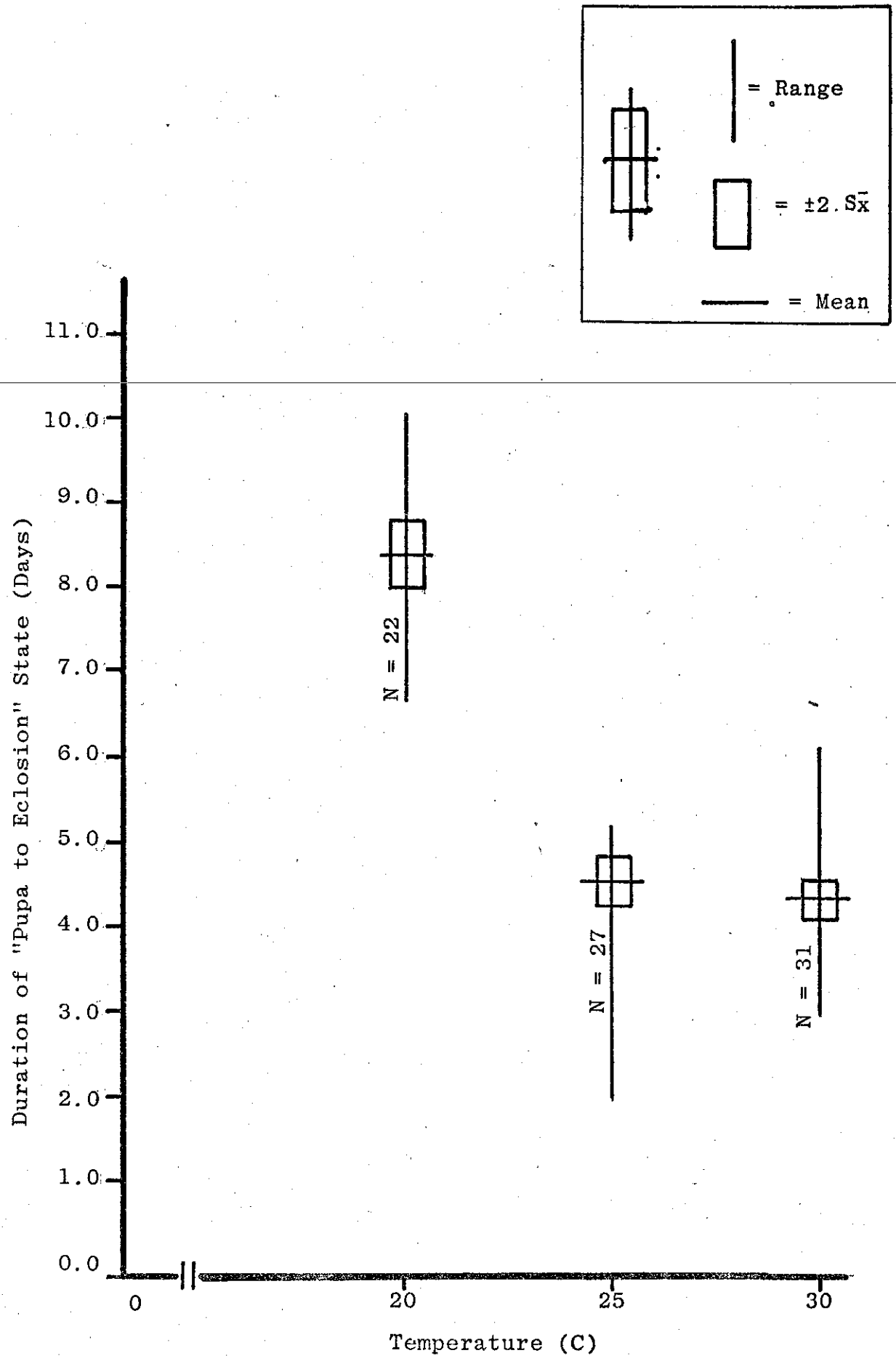

= Range
= ±2.$S_{\bar{x}}$
= Mean
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Duration of "Pupa to Eclosion" State (Days)
N = 22
N = 27
N = 31
0
20
25
30
Temperature (C)

Figure 11. Regression line of duration of "pupa to eclosion" stage at different temperatures in D. nebulosa (Reciprocal transformation of both duration and temperature was necessary.)

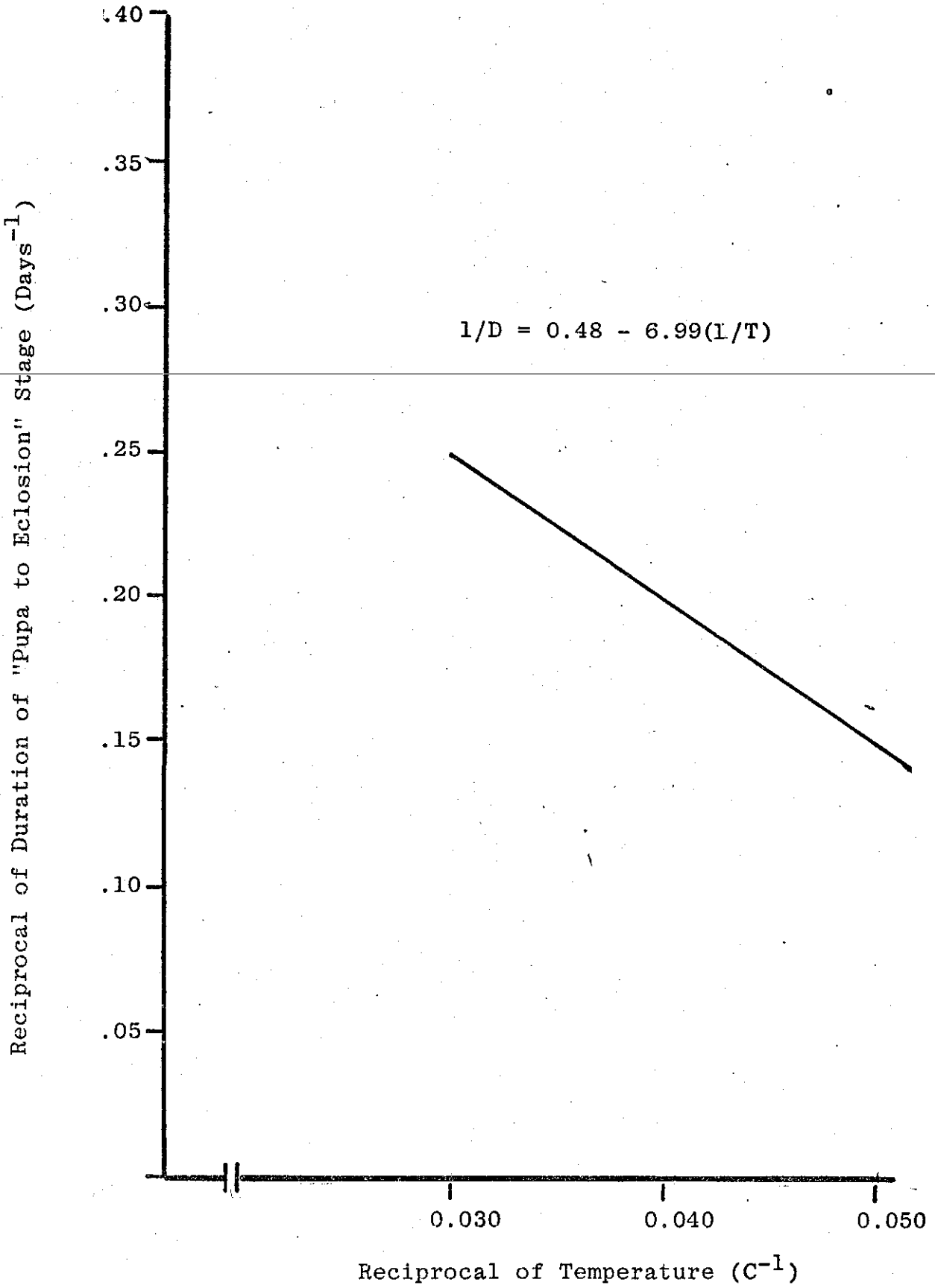
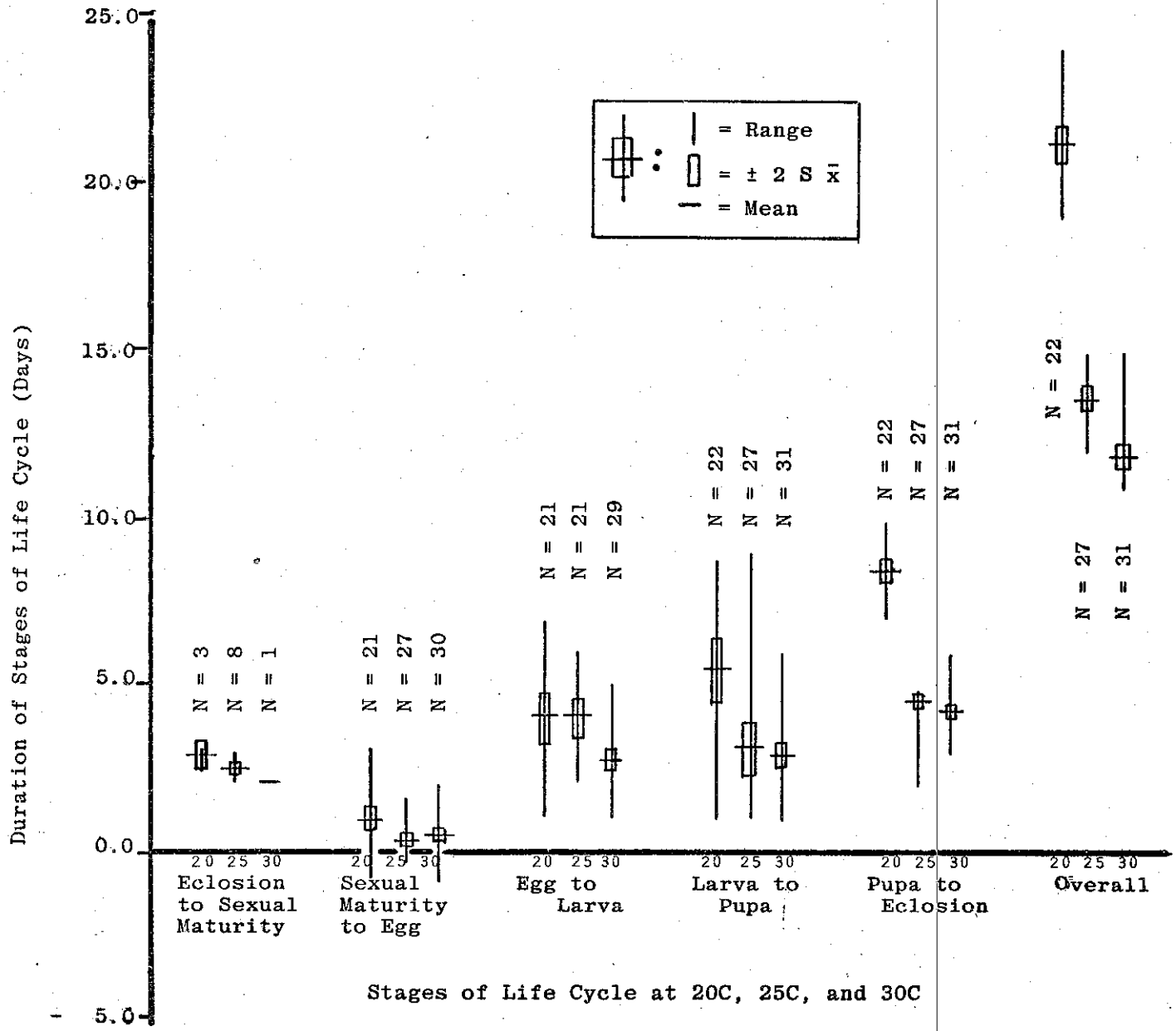

.40
.35
.30
.25
.20
.15
.10
.05
1/D = 0.48 - 6.99(1/T)
Reciprocal of Duration of "Pupa to Eclosion" Stage (Days^{-1})
0.030
0.040
0.050
Reciprocal of Temperature (C^{-1})

Figure 12. Summary of duration of the stages of the life cycle at 20 C, 25 C, and 30 C in D. nebulosa (Mean, range, 2 standard errors of the mean, and N are shown.)


25.0
20.0
15.0
10.0
5.0
0.0
5.0
Duration of Stages of Life Cycle (Days)
= Range
= ± 2 S x̄
= Mean
N = 3
N = 8
N = 1
N = 21
N = 27
N = 30
N = 21
N = 21
N = 29
N = 22
N = 27
N = 31
N = 22
N = 27
N = 31
N = 22
N = 27
N = 31
20 25 30
Eclosion to Sexual Maturity
Sexual Maturity to Egg
Egg to Larva
Larva to Pupa
Pupa to Eclosion
Overall
Stages of Life Cycle at 20C, 25C, and 30C

DISCUSSION

As stated in the introduction, the hypothesis to be tested states that species which live in a wide range of climates (eurytherms) may have a superior capacity for adaptation to different temperatures than do species which live in a narrow range of climates (stenotherms) (Hunter, 1964). The purpose of this experiment is to study the effect of temperature on the life cycle of D. nebulosa and also to learn if this stenothermal species conforms to the hypothesis.

As the results indicate, the duration of each individual stage and consequently, the overall life cycle, generally decreases with increasing temperature. This pattern is typical of poikilothermic animals which rely to a much greater extent upon the heat derived from the environment for the determination of their own body temperature than do homeothermic animals. However, the degree to which individual species depend upon the environmental temperature varies among the poikilotherms. Stenothermal species are somewhat dependent upon the environmental temperature while eurythermal species are relatively independent of the environmental temperature. Therefore, one would expect D. nebulosa to show a more marked decrease in the length of its life

cycle with increasing temperatures in comparison with a eurythermal species which would be expected to decrease less under the same conditions.

As Figure 1 depicts, the decrease in the duration of the overall life cycle between 20 C and 25 C is approximately 4 times greater than that between 25 C and 30 C. This difference could be explained if the optimum temperature of D. nebulosa were between 25 C and 30 C as might be surmised from the geographic distribution. Tantawy and Mallah (1961) found that stenokous populations of Drosophila were better adapted than euryokous populations only to the temperatures similar to that of their native conditions. Therefore, at or near its optimum temperature, D. nebulosa would be more independent of the environmental temperature than at other temperatures, such as the 20 C used in this experiment.

It is of interest to compare these results of D. nebulosa with those of a eurythermal species such as D. melanogaster. Suzuki (1970) reported that the overall life cycle of D. melanogaster required approximately 25 days at 17 C, 11.5 days at 22 C, and 7.5 days at 29 C. Although the interval between 17 C and 22 C is smaller than between 22 C and 29 C, the decrease in the duration in the 17 C-22 C interval is still approximately 3 times greater than that in the 22 C-29 C interval. This change in the decrease could again be possibly explained in terms of the optimum temperature which has been reported by Young and Plough (1926) to be

24 C for D. melanogaster. When the environmental temperature approaches 24 C, the effect of temperature on the length of the life cycle in addition to several other rate functions is not as pronounced as it would be at temperatures further from the optimum, such as 17 C.

It is clear that if one were to extrapolate the values at several temperatures reported in the literature of D. melanogaster to calculate values at 20 C, 25 C, and 30 C, the duration at each temperature would be less than that of D. nebulosa. However, it is not apparent that D. nebulosa displays a marked decrease in duration with increasing temperature when compared with D. melanogaster which would be expected to have a relatively less decrease in duration. In fact, both species appear to follow quite similar patterns of an initial pronounced decrease followed by a relatively smaller decrease as the optimum temperature is approached. Firmer conclusions could be made if both species were studied at the same temperatures.

Van't Hoff's rule mentioned in the introduction states that in an in vitro biochemical system, the rate of a reaction doubles for each 10 C increase in temperature (Gordon, et al., 1972). This is represented as $Q_{10} = 2$, where Q_{10} is the factor by which a reaction velocity is increased for a rise of 10 C. The formula to calculate Q_{10} is the following:

$$Q_{10} = \left(\frac{K_1}{K_2}\right)^{10/(t_1-t_2)}$$

where K_1 and K_2 are velocity constants corresponding to temperatures t_1 and t_2. Q_{10} varies over the temperature range and is higher in low ranges than in high ranges. Hence, the temperature range from which a Q_{10} is calculated must be specified (Prosser, 1973).

Table 4 lists the duration of the life cycle of various Drosophila species including that of D. nebulosa. The duration of the egg to eclosion period is also listed for some species because the length of the overall life cycle was not available in the literature. It is necessary to point out that the duration of the life cycle is measured quantitatively as the number of days to reach an arbitrary stage of development. Biochemical rates generally increase with increasing temperature, and the rate of development in Drosophila is no exception. However, in this experiment, the number of days required for development was measured, and the reciprocal of this is the rate of development.

At a given temperature range, the Q_{10} of a eurythermal species is expected to be less than that of a stenothermal species if it is relatively more independent of the environmental temperature. As Table 5 shows, in the 6 species for which observations were made at 3 different temperatures, the Q_{10} is always greater at the lower range than at the higher range. This would indicate that at the lower temperature range the flies were dependent upon and affected by the environmental temperature to a greater extent than

TABLE 4

Duration of the overall life cycle and/or egg to eclosion period at different temperatures in *Drosophila* species

Species	Temperature (C)	Overall Life Cycle (days)	Egg to Eclosion (days)	Reference
D. nebulosa	20	21.1	17.8	(This paper)
	25	13.6	11.7	(This paper)
	30	11.9	9.7	(This paper)
D. melanogaster	17	25.0	----	Suzuki, 1970
	22	11.5	----	Suzuki, 1970
	29	7.5	----	Suzuki, 1970
D. virilis	15	34.0	27.0	Hunter, personal communication
	25	21.0	13.0	de Flores, 1976
D. viracochi	15	38.0	27.0	Hunter, personal communication
	20	27.0	17.3	de Flores, 1976
D. immigrans	15	31.0	25.0	Hunter, personal communication
	25	14.9	11.1	de Flores, 1976
D. pseudoobscura	16	----	32.5	Ray, 1960
	19	----	19.0	Ray, 1960
	24	----	18.0	Ray, 1960
D. persimilis	16	----	32.5	Ray, 1960
	19	----	19.0	Ray, 1960
	24	----	18.0	Ray, 1960
D. willistoni	16	----	32.5	Ray, 1960
	19	----	19.0	Ray, 1960
	24	----	10.5	Ray, 1960
D. equinoxialis	16	----	32.5	Ray, 1960
	19	----	19.0	Ray, 1960
	24	----	10.5	Ray, 1960
D. pavani	16	----	40.2	Budnik, et al., 1971
	25	----	18.8	

TABLE 5

Q_{10} of the overall life cycle and/or egg to eclosion period for different temperature ranges in Drosophila species

Species	Temperature Range (C)	Q_{10} of Overall Life Cycle	Q_{10} of Egg to Eclosion Period
D. nebulosa	20-25	2.4	2.3
	25-30	1.3	1.4
	20-30	1.8	1.8
D. melanogaster	17-22	4.8	---
	22-29	1.8	---
	17-29	2.7	---
D. virilis	15-25	1.6	2.1
D. viracochi	15-20	2.0	2.4
D. immigrans	15-25	2.1	2.3
D. pseudoobscura	16-19	---	6.2
	19-24	---	1.1
	16-24	---	2.1
D. persimilis	16-19	---	6.2
	19-24	---	1.1
	16-24	---	2.1
D. willistoni	16-19	---	6.2
	19-24	---	3.3
	16-24	---	4.2
D. equinoxialis	16-19	---	6.2
	19-24	---	3.3
	16-24	---	4.2
D. pavani	16-25	---	2.3

at the higher range which most likely also included the optimum temperature. It is also interesting to note that D. virilis at a lower temperature range, 15 C-25 C, still had a lower Q_{10} for the overall life cycle than D. nebulosa at a higher temperature range of 20 C-30 C. This implies that D. virilis is better adapted to lower temperatures than is D. nebulosa. Perhaps it would be found that D. virilis, a eurythermal species, is also better adapted to higher temperatures than is D. nebulosa, but this data was not available. The Q_{10}'s of the egg to eclosion period of D. nebulosa at 20 C-25 C and of D. pavani at 16 C-25 C are equal despite the fact that D. pavani's range is larger. This implies that D. pavani has a greater capacity to adapt to lower temperatures than does D. nebulosa. Similar implications can be made when comparing the Q_{10}'s of the overall life cycle of D. nebulosa at 20 C-25 C with that of D. immigrans at 15 C-25 C. The Q_{10} of D. immigrans is still less than that of D. nebulosa even though the former species' range is greater. D. immigrans is eurythermal, so these results are not surprising. Lastly, the Q_{10} of the overall life cycle of D. viracochi at 15 C-20 C is less than that of D. nebulosa at a higher range of 20 C-25 C. D. viracochi is a stenothermal species as is D. nebulosa, so these results would imply only that D. viracochi is better adapted to lower temperatures. Comparisons of the Q_{10}'s of D. nebulosa with those of D. melanogaster, D. pseudoobscura, D. persimilis,

D. willistoni, and D. equinoxialis would not be feasible with the given data due to the inconsistency of the temperature ranges. The comparisons of the Q_{10}'s which were discussed imply that the stenothermal species, D. nebulosa, does conform to the hypothesis, but again, no firm conclusions can be made because the temperatures at which the other species were observed differ from those of D. nebulosa and also because the optimum temperature is not known.

In the second portion of these experiments, the resistance of D. nebulosa to a cold temperature of 15 C was studied. When virgin males and females from 25 C were placed together in experimental vials at 15 C, eggs were laid by the females after a long period. Since these eggs never produced larvae but instead, soon degenerated, it was concluded that the eggs were either unfertilized or not viable at this low temperature. When eggs, larvae, and pupae were transferred from 25 C to 15 C, the percentage of emergence was only approximately 50%. According to Powsner (1935), some developmental processes probably always occur at temperatures at which the organism is not killed by the cold. Also, some of these processes may be slowed up more at low temperatures than others are. After a period this must cause "disorganization" of development to such an extent that some time must elapse, when the organism is raised to a higher temperature, before the processes become sufficiently correlated for development to proceed normally again. If the "disorganization" has

gone too far, the organism cannot complete development (Powsner, 1935). This and the fact that D. nebulosa normally lives in a warm environment could account for the very low survival rate at the low temperature. Perhaps, the results would have been different if the flies, eggs, larvae, and pupae were transferred from 20 C instead of 25 C. This could possibly lessen the effects of temperature shock.

The first and major portion of these experiments was to study the effects of temperature on the life cycle of D. nebulosa. If this experiment were to be repeated, a few changes in the procedure should be made to provide more comparable results. The flies which were used to measure the time when sexual maturity was reached and mating occurred should also be used in the remaining portion of the experiment where the different stages of the life cycle were noted. This would decrease the individual variability among the flies. In this experiment, the time of the first appearance of eggs, larvae, pupae, and emerging adults was recorded. Budnik, et. al. (1971) worked with D. pavani and found that at every temperature studied, 2 groups were established according to their emergence: those of "fast" development (25 to 35 days at 16 C, 14 to 16 days at 25 C), and those of "slow" development (44 to 65 days at 16 C, and 21 to 29 days at 25 C). Consequently, with the method used in this experiment, the existence of a slower developing group would not be detected. Therefore, it would be more accurate to note

the range of days during which a particular stage appeared. This would require transferring the flies to new vials continually. Also, since virgin males and females were captured and placed together every 12 hours, the experimental vials should have been examined for offspring also every 12 hours instead of every 24 hours as was done in this experiment. This consistency would provide more accurate data. As stated in the Materials and Methods section, green food coloring was added to each experimental vial to provide a contrasting background for the light-colored eggs. However, the larvae are still difficult to see, especially the small instar stages. Unless the larvae forage on the surface of the food or on the walls of the vials, they are almost impossible to detect. In several experimental vials, larvae were first seen on the same day as pupae, and sometimes larvae were not seen at all. Both situations are represented in the results by a smaller N value for the stage, egg to larva. A rarer situation which occurred was when eggs were first seen on the same day that larvae were seen, and this is also represented in the results by a smaller N value for the sexual maturity to egg stage. These problems of seeing eggs and larvae could be remedied by the use of Drosophila food that is more transparent. Another method would be to have the flies lay their eggs on a flat surface in the vial with no depth of food (Hunter, personal communication). Complications also arose when sterile foam rubber instead of sterile cotton was

used as stoppers for the experimental vials in the measurement of the "eclosion to sexual maturity" stage at 30 C. The media of the majority of the vials were contaminated by some type of microorganism which probably passes through foam rubber more readily than through cotton. As a result, progeny were found in only one vial which was far less than what was expected. No regression line for the "sexual maturity to egg" stage could be calculated using the common transformation methods such as reciprocal, log, or arcsin transformations. Since the values at 30 C were derived from the single value of the "eclosion to sexual maturity" stage, and since a sample size of one gives no estimate of the variability, the calculation of a line of regression was not further pursued.

It would be interesting to study the effects of temperature on the respiration of D. nebulosa to obtain a better idea of how this species conforms with the hypothesis concerning adaptation. An experiment similar to that of Hunter (1964, 1965, 1966, 1968) could be conducted in which the rate of oxygen consumption of flies grown at a high and low temperature is measured at an intermediate temperature. It would be expected that the flies acclimated at the high temperature would have the same or a greater rate of oxygen consumption than those acclimated at the low temperature which according to Prosser and Brown (1961), is not the normal pattern of temperature adaptation. Therefore, this result would be in agreement with the hypothesis.

Also, the extent to which Bergmann's and Allen's Rules apply to D. nebulosa could be studied. To reiterate, Bergmann's Rule states that the body size of geographical races of a species is smaller in the warmer parts of the species' range than in the colder parts of the range. Allen's Rule states that protruding body parts are relatively shorter in the colder parts of the range of a species than in the warmer parts. A procedure similar to that of Ray (1960) could be performed in which flies are grown at 2 different temperatures, and measurements of wing and leg length as well as overall body size are compared. If the changes suggested by Bergmann's and Allen's Rules occur in this species, this might be considered a type of temperature adaptation. Comparisons of stenothermal and eurythermal poikilotherms have not yet been made with respect to these rules.

SUMMARY

The effect of temperature on the life cycle of Drosophila nebulosa is studied in this experiment. The mean duration of the overall life cycle is 21.1±0.3 days at 20 C, 13.6±0.2 days at 25 C, and 11.9±0.2 days at 30 C. Formulas which allow the prediction of the duration of the various stages of the life cycle at any temperature are derived from regression lines.

The hypothesis that is tested in this experiment states that species which live in a wide range of climates (eurytherms) may have a superior capacity for adaptation to different temperatures than to species which live in a narrow range of climates (stenotherms) (Hunter, 1964). When the Q_{10}'s of D. nebulosa, a stenothermal species, are compared with those of eurythermal species, the results indicate that D. nebulosa does conform to this hypothesis.

LITERATURE CITED

Alpatov, W. W. 1932. Egg production in Drosophila melanogaster and some factors which influence it. J. Exp. Zoo. 63: 85-111.

Alpatov, W. W. and R. Pearl. 1929. Influence of temperature during the larval period and adult life on the duration of the life of the imago of Drosophila melanogaster. Amer. Natur. 63: 37-67.

Budnik, M., S. Koref-Santibanez, and D. Brncic. 1971. Rate of development and inversion polymorphism in Drosophila pavani at two temperatures. Genetics 69: 227-233.

Burr, M. J., and A. S. Hunter. 1969. Effects of temperature on Drosophila - V. Weight and water, protein and RNA content. Comp. Biochem. Physiol. 29: 647-652.

Burr, M. J., and A. S. Hunter. 1970. Effects of temperature on Drosophila - VII. Glutamate-asparate transaminase activity. Comp. Biochem. Physiol. 37: 251-256.

Crumpacker, D. W., and D. Marinkovic. 1967. Preliminary evidence of cold temperature resistance in Drosophila pseudoobscura. Amer. Natur. 101: 505-514.

David, J., M. F. Arens, and Y. Cohet. 1971. Male sterility in Drosophila melanogaster at a high temperature; Nature, course and reversibility of the effects of heat. Biological Abstracts 70006-93342. Vol. 52.

de Flores, M. M. M. 1976. The life cycle of seven species of Drosophila. M. S. Thesis. Univ. of the Pacific (Libr. Univ. Card No. QL537-D76-F63). 34 pp.

Druger, M. 1962. Development of D. pseudoobscura at low temperature. Drosophila Information Service 36: 63.

Flagg, R. O. 1973. Carolina Drosophila Manual. Carolina Biological Supply Company, North Carolina. 23 pp.

Garcia, P. C., and O. M. Suarez. 1962. Especies del genero Drosophila encontradas hasta ahora en Venezuela. Revista Venezolana de Sanidad y Asistencia Social XXVII: 2.

Gordon, M. S., G. A. Bartholomew, A. D. Grinnell, C. B. Jorgensen, and F. N. White. 1972. Animal Physiology: Principles and Adaptations. Macmillan Company, New York. 592 pp.

Heed, W. B. 1956. Apuntes sobre la ecologia y la dispersion de los Drosophilidae (Diptera) de El Salvador. Sobretiro de "Comunicaciones" del Instituto Tropical de Investigaciones Cientificas de la Universidad de El Salvador. pp. 59-74.

Hunter, A. S. 1964. Effects of temperature on Drosophila - I. Respiration of D. melanogaster grown at different temperatures. Comp. Biochem. Physiol. 11: 411-417.

Hunter, A. S. 1965. Effects of temperature on Drosophila - II. Respiration of D. pseudoobscura and D. viracochi grown at different temperatures. Comp. Biochem. Physiol. 16: 7-12.

Hunter, A. S. 1966. Effects of temperature on Drosophila - III. Respiration of D. willistoni and D. hydei grown at different temperatures. Comp. Biocehm. Physiol. 19: 171-177.

Hunter, A. S. 1968. Effects of temperature on Drosophila - IV. Adaptation of D. immigrans. Comp. Biochem. Physiol. 24: 327-333.

Hunter, A. S., and A. Navarro. 1969. A Natural Population of Drosophila (Diptera: Drosophilidae) in Columbia. Ann. Entomol. Soc. Amer. 62 (5): 1101-1105.

Lauge, G. 1972. Life span and reproduction rate of Drosophila subjected to elevated temperatures. Biological Abstracts 46689-70028. Vol. 55.

Levins, R. 1969. Thermal acclimation and heat resistance in Drosophila species. Am. Nat. 103: 483-499.

Northrop, J. H. 1920. Concerning the Hereditary Adaptation of organisms to higher temperature. J. Gen. Physiol. 2: 313-318.

Pantelouris, E. M. 1957. Size response of developing Drosophila to temperature changes. J. Genetics 55: 507-510.

Patterson, J. T. 1943. Studies in the genetics of Drosophila - III. The Drosophilidae of the Southwest. Univ. Texas Pub. 4313: 69-70.

Powsner, L. 1935. The effects of temperature on the durations of the developmental stages of Drosophila melanogaster. Physiol. Zool. 8 (4): 474-520.

Prosser, C. L. 1973. Comparative Animal Physiology. W. B. Saunders Company, Philadelphia. pp. 362-428.

Prosser, C. L., and F. A. Brown, Jr. 1961. Comparative Animal Physiology. W. B. Saunders Company, Philadelphia. pp. 238-284.

Ray, C. 1960. The application of Bergmann's and Allen's Rules to the poikilotherms. J. Morph. 106: 85-108.

Simpson, G. G., A. Roe, and R. C. Lewontin. 1960. Quantitative Zoology. Harcourt, Brace and Company, New York. 440 pp.

Smith, J. M. 1958. The effects of temperature and of egg-laying on the longevity of Drosophila subobscura. J. Exp. Biol. 35: 832-842.

Spieth, H. T., and W. B. Heed. 1972. Experimental systematics and ecology of Drosophila. Annual Review of Ecology and Systematics 3: 269-284.

Suzuki, D. T. 1970. Temperature-sensitive mutations in Drosophila melanogaster. Science 170: 695-706.

Tantawy, A. O., and G. S. Mallah. 1961. Studies on natural populations of Drosophila - I. Heat resistance and geographical variation in Drosophila melanogaster and D. simulans. Evolution 15: 1-14.

Young, W. C., and H. H. Plough. 1926. On the sterilization of Drosophila by high temperature. Biol. Bull. 51: 189-198.